ORIGINAL ARTICLE

Risk Factors Associated with the Presence of Viable *Listeria monocytogenes* in Bulk Tank Milk from US Dairies

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Impacts

- In the United States, dairy farms with more than 500 milking cows are five times more likely to have bulk tank milk (BTM) contamination with *Listeria monocytogenes* than are smaller dairies with <100 milking cows.
- Dairy farms located in the southeast and northeast of the United States are at a higher risk of having BTM contaminated with *L. monocytogenes* than are farms located in the western USA.
- An operations' risk of BTM contamination with *L. monocytogenes* appears to vary according to the geographical location and the management practices prevalent in that specific region.

Keywords:
Listeria monocytogenes; bulk tank milk; risk factors

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Summary

The objective of the study was to screen a large number of herd management practices and herd characteristics from US dairies to identify herd-level risk factors associated with the presence of *Listeria monocytogenes* in bulk tank milk (BTM). A total of 71 variables was univariately evaluated for their association with the presence of *L. monocytogenes* in BTM. Results from the univariate analysis indicated that using automatic take offs and having an open herd management increased the risk of BTM contamination with *L. monocytogenes*, while storing manure in outside pens not accessible to cattle decreased the risk. These variables, however, were not sustained in the multivariable model, which indicated that the presence of *L. monocytogenes* in BTM was significantly associated with region of the country (farms in the southeast and northeast were six and four times more likely respectively, to have BTM contamination than farms in the west) and number of milking cows (herds with >500 milking cows were five times more likely to have BTM contamination than herds with <100 milking cows). In conclusion, our results suggest that risk factors associated with BTM contamination are different depending on the geographical region and herd size of the operation.

Introduction

Listeria monocytogenes is a saprophytic environmental bacterium that causes listeriosis, a severe disease of animals and humans. Listeriosis is a food-borne disease in humans which mainly affects pregnant women, neonates, elderly and immunocompromised individuals. Clinical listeriosis in cattle is characterized by depression, limb weaknesses, difficulty in swallowing, walking in circles, droopy lower lip, mastitis and abortion during the last trimester of gestation. The prevalence of *L. monocytogenes* in raw milk worldwide was estimated to be approximately 2.2% (Farber and Peterkin, 1991). Regional surveys in the USA have reported the presence of *L. monocytogenes* in bulk tank milk (BTM) in 4.6% of the farms in eastern South Dakota and western Minnesota and in 12.6% of the milkline filters from New York dairy farms (Hassan et al., 2000; Jayarao and Henning, 2001). A study conducted as part of the National Animal Health Monitoring System (NAHMS) dairy 2002 survey indicated that

L. monocytogenes was present in 6.5% of BTM on US dairy farms (Van Kessel et al., 2004).

The importance of *L. monocytogenes* to the dairy industry was well understood by food manufacturers and regulatory agencies after several outbreaks of listeriosis in humans were linked to the consumption of dairy products. In Europe, more than half of the outbreaks and some of the sporadic cases of human listeriosis were linked specifically to the consumption of raw milk and milk-derived products (Lunden et al., 2004). A 1985 California outbreak, which had the highest morbidity and mortality rates of all listeriosis outbreaks in the USA, was associated with the consumption of Mexican-style soft cheese (Linnan et al., 1988).

The contamination of BTM with *L. monocytogenes* was previously found to be associated with poor cow and barn cleanliness, poor hygiene during the milking process and absence of good milking practices (Husu et al., 1990; Sanaa et al., 1993; Hassan et al., 2001). These studies, however, looked at risk factors associated with the presence of *L. monocytogenes* in BTM at a regional level in the USA or in other countries which might not represent the variety of herd sizes and management practices present at a national level in the USA.

The transmission dynamics and the ecology of *L. monocytogenes* in animal production environments are extremely complex and not fully understood partly because *L. monocytogenes* can be found in a wide variety of plants, soil and faeces from wild animals and birds. This study was based on the same set of data as the one by Van Kessel et al. (2004), in which the authors determined the prevalence of *L. monocytogenes* and other human pathogens in BTM. The objective of this study, however, was to screen a representative sample of US dairy operations to evaluate herd management practices and herd characteristics to identify herd-level risk factors associated with the presence of *L. monocytogenes* in BTM. The potential identification of new risk factors associated with the prevalence of *L. monocytogenes* in BTM nationwide could contribute to implementing more comprehensive management practices needed to prevent BTM contamination with this pathogen.

Materials and Methods

Survey and BTM samples

A stratified random sample based on herd size from each of 21 states was selected from a sample list frame provided by the USDA, National Agricultural Statistics Service (USDA–NASS) to represent 81% of the US dairy operations and 85% of the US dairy cow population. Producers reporting one or more milk cow in their inventory by 1 January 2002 were included in Phase I of the NAHMS 2002 dairy study. During Phase I, 2461 of 3876 eligible operations responded to a general management questionnaire administered by USDA–NASS. Only 2343 operations that participated in Phase I and had 30 or more milking cows were eligible to participate in Phase II. Of the 1438 that consented to Phase II, 1013 producers agreed to participate and completed the second survey.

Between 25 February and 30 June 2002, animal health officials collected a single BTM sample from each of 871 dairy operations with at least 30 milking cows in the 21 selected states. BTM samples were only collected when at least 70% of the herd's lactating cows were represented in the sample. Characteristics of collection, samples and laboratory tests are detailed elsewhere (Van Kessel et al., 2004). The questionnaires covered a variety of topics regarding general management, animal health, herd characteristics, handling of manure and waste treatment, milking procedures, biosecurity and cattle inventory. Details regarding design of the survey are described elsewhere (USDA 2002, 2003).

Analysis of risk factors associated with the presence of *L. monocytogenes* in BTM

The survey design was a stratified random sample with unequal selection probabilities in each stratum. The unequal selection probabilities were implemented to ensure that large dairy operations were well represented in the sample. Weights were created for each operation to account for the selection probabilities and then adjusted for non-response. Complete details about this complex survey design are published elsewhere (USDA 2002–2003). All statistical analyses accounted for both the complex survey design and the individual farm weights. The outcome variable was the dichotomous result of the laboratory culture of milk for the presence of *L. monocytogenes*.

Complete data for test results from BTM and management practices were available from 850 dairy farms. The association between the presence of *L. monocytogenes* in BTM and 71 *a priori* identified management practices and characteristics of the operations (Table 1) were evaluated univariately with a log-likelihood chi-squared test using PROC CROSSTAB which accounts for the complex study design and sampling weights by using a Taylor series approximation to estimate variance (SUDAAN, Release 8.0; Research Triangle Institute, Research Triangle Park, NC, USA). The variables were selected based on: (i) prior evidence of their association with the presence of *L. monocytogenes* in raw milk; (ii) prior knowledge about their biological role in the ecology of listeriosis; (iii) their indirect association with factors that are part of the epidemi-

Table 1. Herd characteristics and management practices that met the selection criterion ($P < 0.25$) for inclusion in PCCA after the univariate analysis

Variable	Category	% of US dairies	% dairies with Listeria monocytogenes in BTM	Univariate analysis	
				Log-likelihood chi-square	P-value
Herd characteristics					
Herd size (# of milking cows)[a]	<100	38.9	3.6	4.91	0.087
	100–500	39.6	7.0		
	>500	21.5	10.0		
	All	100.0	6.5		
Region of the country[a]	West[b]	20.0	3.5	5.17	0.161
	Midwest[c]	42.1	2.5		
	Northeast[d]	30.4	10.0		
	Southeast[e]	7.5	11.0		
Feed types					
Corn silage[a]	Yes	84.7	7.3	1.48	0.223
	No	15.3	2.3		
Cotton seed meal[a]	Yes	11.1	1.8	1.87	0.172
	No	88.9	7.2		
Wheat-not silage[a]	Yes	9.4	11.7	2.89	0.090
	No	90.6	6.1		
Milking procedures					
Use of backflush system[a]	Yes	10.8	4.6	1.43	0.232
	No	89.2	6.8		
Use of automatic take offs[f]	Yes	60.1	9.2	3.88	0.049
	No	39.9	2.8		
General herd management practices					
Bedding: sand[a]	Yes	21.0	6.2	2.57	0.110
	No	79.0	6.7		
Floor surface moisture in the summer[a]	Dry	50.5	5.1	5.66	0.060
	Half time wet	23.4	3.8		
	Always wet	26.1	11.9		
Floor surface moisture in the winter[a]	Dry	32.8	4.0	2.88	0.237
	Half time wet	28.5	4.2		
	Always wet	38.7	10.6		
Administration of bST[a]	Yes	37.4	9.6	3.40	0.066
	No	63.4	4.6		
Cattle brought to the operation[f]	Yes	55.8	9.0	4.51	0.034
	No	44.2	3.4		
Health management					
Cattle dying with neurological signs[a]	Yes	8.1	7.4	1.71	0.191
	No	91.9	6.7		
Vaccination against coliform mastitis[a]	Yes	48.5	9.1	3.36	0.067
	No	51.5	3.9		
Manure handling and waste management					
Manure left on pasture in cow areas[a]	Yes	61.0	5.7	2.48	0.116
	No	39.0	7.9		
Use of gutter cleaner in cow areas[a]	Yes	32.3	3.4	1.79	0.181
	No	67.7	8.1		
Dry lot scraped in cow areas[a]	Yes	57.6	4.5	1.46	0.227
	No	42.4	9.4		
Waste stored in manure spreader[a]	Yes	35.0	5.1	1.62	0.204
	No	65.0	7.3		
Untreated liquid manure stored in earth-basin[a]	Yes	38.1	9.4	1.76	0.184
	No	61.9	4.8		
Outside storage for solid manure not in dry lot or pen[f]	Yes	37.0	4.1	6.11	0.014
	No	63.0	7.9		
Manure applied to forage to be ensiled[a]	Yes	37.6	10.1	3.08	0.079
	No	62.4	5.0		

PCCA, principal component cluster analysis; BTM, bulk tank milk.
[a]Variables statistically significant at $P < 0.25$ and subjected to PCCA. [b]West (California, Colorado, Idaho, New Mexico, Texas and Washington). [c]Midwest (Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio and Wisconsin). [d]Northeast (New York, Pennsylvania and Vermont). [e]Southeast (Florida, Kentucky, Tennessee and Virginia). [f]Variables statistically significant at $P < 0.05$ in the univariate analysis.

ology of listeriosis in animals and (iv) the idea that, even although no prior link with *L. monocytogenes* was reported or seemed intuitive for some variables, it was worth exploring the available data and its potential link with the presence of *L. monocytogenes* in BTM in the USA. The variables evaluated in the univariate analysis were categorized into six groups: (i) herd-level characteristics, (ii) feed types, (iii) milking procedures, (iv) general herd management practices, (v) health management and (vi) manure handling and waste treatment methods.

Variables with log-likelihood chi-squared test values of $P < 0.25$ were subjected to principal component cluster analysis (PCCA) as a variable reduction procedure. PCCA was conducted using PROC VARCLUS in SAS version 9.1 (SAS Institute Inc, Cary, NC, USA, 2002–2003). This procedure allows for the formation of clusters of variables that are as correlated as possible among themselves and as uncorrelated as possible with variables in other clusters. The maximum eigenvalue used for cluster analysis was 1.0. The selection of the representative variable from each cluster for inclusion in the multivariate model was based on the lowest $1-R^2$ ratio ($1-R^2$ with own cluster/$1-R^2$ with next closest cluster). A variable typical of its cluster is expected to have a ratio close to zero, while a variable highly correlated with other clusters is expected to have a ratio close to one. A backward elimination approach was used to build the multivariable logistic regression model using PROC RLOGISTIC (SUDAAN) with all the variables selected after PCCA. Observations with missing values for any of the independent variables were not included in the model building, thereby reducing the number of observations from 850 to 726. Region and herd size were not included in the cluster analysis because we wanted to examine their role as confounders and effect modifiers in the construction of the final model. The final model included the complete dataset with 850 observations and only variables with Wald-*F* statistic values of $P < 0.05$.

All variables selected after the PCCA were monitored for their potential action as confounding variables by comparing the crude and adjusted odds ratios (OR) obtained with the full and the reduced model respectively. Differences between the crude and the adjusted OR > 10% were attributed to the confounding effects of the added variable. Confounding factors were included in the final model regardless of their *P*-value. The goodness-of-fit for the final multivariable model was assessed with the Hosmer and Lemeshow goodness-of-fit test.

Results

The prevalence of *L. monocytogenes* in BTM samples from US dairies reported by Van Kessel et al. (2004) was 6.5%. Twenty-two variables within the categories of herd characteristics, feed type, milking procedures and general herd management met the criteria for inclusion in the development of the multivariate model as a result of the univariate analysis (Table 1). Three of the 22 variables (use of automatic take offs, outside storage of solid manure – not in dry lot or pen and bringing cattle onto the operation) showed a statistically significant association with the presence of *L. monocytogenes* in BTM with a *P*-value < 0.05. The use of automatic take offs and bringing new additions to the operation were found to be associated with an increased risk of BTM contamination with *L. monocytogenes*, while storing manure in outside pens not accessible to cattle was associated with a lower risk of BTM contamination ($\alpha = 0.05$).

Principal component cluster analysis reduced the 22 variables which met the modelling criteria to seven uncorrelated variable clusters (Table 2). The seven clusters resulting from this PCCA explained 52% of the variation of the variables included. Clusters 2 and 7 represented manure handling and manure storage methods and Cluster 3 represented feedstuffs. The remaining clusters could not be easily described because they were constituted by variables representing a variety of management practices. The heterogeneity among their constituent variables was reflected in a low proportion of explained variation.

The final model displayed in Table 3 only included herd size ($P = 0.02$) and region ($P = 0.06$). Approximately, 72% of large operations was in the West region. The majority of small operations were in the Midwest region (63.2%) and 48.3% of medium herds was located in the Midwest. Region was included in the final model because of the variable's effect on the measured association between herd size and the presence of *L. monocytogenes* in BTM. Region acted as a confounder for herd size by causing a change between the crude and adjusted OR > 40%. An interaction term between region and herd size was explored in the final model and found to be not statistically significant. Farms located in the southeast and those located in the northeast were 6.24 and 4.33 times more likely respectively, to have BTM contamination with *L. monocytogenes* than were farms located in the west. An association was also found between herd size and the presence of *L. monocytogenes* in milk. Large dairies (>500 cattle) were 5.05 times more likely to have *L. monocytogenes* in BTM than are farms with <100 head. The Hosmer and Lemeshow goodness-of-fit test indicated good model fit ($P = 0.73$).

Discussion

A major strength of this study is the national scope of the sampling and the availability of abundant information regarding herd characteristics and management practices,

Table 2. Results of principal components cluster analysis using proc varclus in sas for selection of variables included in the univariate analysis

Cluster no.	Variable	Proportion of variation explained[a]	$1-R^2$ ratio[b]
1	Surface moisture in winter[c]	0.18	0.29
	Bedding: sand		0.92
	Surface moisture in summer		0.42
	Use of automatic take offs		0.64
	Use of gutter cleaner in cow areas		0.49
2	Manure left on pasture in cow areas[c]	0.24	0.34
	Manure applied to forage to be ensiled during growing season		0.34
3	Cotton seed meal[c]	0.30	0.49
	Corn silage		0.50
	Wheat-not silage		0.71
4	Administration of bST[c]	0.36	0.49
	Vaccination against coliform mastitis		0.58
	Tail docking		0.70
	Cattle brought to the operation		0.79
5	Dry lot scraped in cow areas[c]	0.42	0.44
	Cattle dying with neurological signs		0.45
6	Use of backflush systems[c]	0.47	0.45
	Outside storage for solid manure not in dry lot or pen		0.45
7	Waste stored in manure spreader[c]	0.52	0.38
	Slurry or liquid manure stored in earth-basin and NOT treated		0.39

[a]This proportion indicates that how well the variables are represented by the cluster.
[b]$1-R^2$ ratio = $1-R^2$ with own cluster/$1-R^2$ with next closest cluster.
[c]Variables within a cluster selected for the multivariate analysis.

Table 3. Multivariable analysis of risk factors and confounders associated with the presence of *Listeria monocytogenes* in BTM from US dairies (n = 850)

Variable	Variable categories	Coefficients	*P*-value	Odds ratio	95% confidence interval
Intercept	–	–4.55	0.09	0.01	0.002–0.04
Herd size[a]	<100	Reference			
	100–500	0.66	0.09	1.94	0.90–4.18
	>500 head	1.62	0.01	5.05	1.63–15.65
Region[b]	West[c]	Reference			
	Midwest[d]	0.75	0.30	2.11	0.52–8.63
	Northeast[e]	1.47	0.04	4.33	1.07–17.55
	Southeast[f]	1.83	0.02	6.24	1.33–29.25

BTM, bulk tank milk.
[a]Overall *P*-value = 0.02.
[b]Overall *P*-value = 0.06.
[c]West (California, Colorado, Idaho, New Mexico, Texas and Washington).
[d]Midwest (Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio and Wisconsin).
[e]Northeast (New York, Pennsylvania and Vermont).
[f]Southeast (Florida, Kentucky, Tennessee and Virginia).

which allowed exploring a wide variety of factors potentially associated with the presence of *L. monocytogenes* in BTM on US dairy farms. Previous studies exploring similar associations were conducted for specific geographical regions and for a limited number of risk factors known to be biologically related to the bacterium (Husu et al., 1990; Sanaa et al., 1993; Hassan et al., 2001, 2002). One recently published study described the prevalence of and risk factors for *Listeria* spp. in BTM in Galicia Spain, but the reported risk factors were not specific for *L. monocytogenes* (Vilar et al., 2007). This study initially explored 71 herd characteristics and management factors via univariate analysis, after which, a variable reduction procedure, PCCA, was used to select the variables that participated

in the construction of the final model. Reducing the number of variables that were found to be associated with the presence of *L. monocytogenes* in BTM by using PCCA was critical in preventing overfitting and model instability. Furthermore, PCCA reduced the likelihood of introducing bias by not allowing highly correlated variables in the final model.

Our results, however, differ from others published previously. Several reasons may account for these differences. Due to the cross-sectional design of this study, data on risk factors and the presence of *L. monocytogenes* in BTM were collected at the same time, which limited our ability to establish causal associations. In addition, basing the analysis on a one-time sample collection did not allow evidencing any dynamic changes that might have occurred in the prevalence levels of *L. monocytogenes* in BTM. Consequently, it is possible that this study missed potential associations between risk factors and the presence of the bacterium in BTM because the study samples were collected during the spring months and not during the winter when the prevalence of the bacterium in milk has been reported to reach its highest levels (Lovett et al., 1987; Liewen and Plautz, 1988; Husu et al., 1990). Participation in all aspects of this study was voluntary. There was potential for selection bias, for example, if producers who knew their status was positive tended to not participate in this study. However, risk factor relationships would not necessarily be affected by this type of selection bias. Sampling weights were adjusted for non-response to help account for potential bias. Although a longitudinal design would have been more appropriate to identify risk factors associated with the pathogen in BTM, it is almost impossible to conduct such study in terms of manpower and funding at the national level.

Previous studies indicated that the contamination of raw milk with *L. monocytogenes* can originate from the environment, the milking system or cows with *Listeria* mastitis (Husu et al., 1990; Sanaa et al., 1993; Hassan et al., 2001). A cross-sectional study conducted in New York reported that pre-milking practices such as forestripping and teat sanitation (e.g. pre-dipping) were associated with a lower risk of BTM contamination with *L. monocytogenes* (Hassan et al., 2001). In this study, however, pre-dipping and forestripping were not associated with the risk of BTM contamination with *L. monocytogenes*. The only milking-related factor found to be univariately associated with an increased risk of BTM contamination with *L. monocytogenes* was the use of automatic take offs. The use of automatic take offs, however, was not significant in the multivariable model.

Faecal material containing *L. monocytogenes* is also considered a potential source of raw milk contamination (Husu, 1990; Yoshida et al., 1998). Faeces containing *L. monocytogenes* might contaminate the udder, the bedding or surfaces where cows lie down or the cows' feed; all of which may lead to BTM contamination if proper milking hygiene protocols are not followed. Several variables related to manure management and storage were explored in this study to determine their impact on the risk for BTM contamination with *L. monocytogenes*. The outside storage of solid manure – not in dry lot or pen – was found to reduce the risk of BTM contamination with *L. monocytogenes* in the univariate analysis. The interpretation of this finding is not readily understood but it could be reflecting a lower level of cow exposure to contaminated manure than cows from farms where manure is stored in pens where cattle have access to stored manure. This variable was not associated with the risk of *L. monocytogenes* in BTM after adjusting for other factors in the final analysis.

Factors that increase the risk of faecal shedding of *L. monocytogenes* could also increase the risk of BTM contamination with the bacterium. Type of feed and feedstuff quality and storage were reported as risk factors for the presence of *L. monocytogenes* in dairy farms (Wiedmann et al., 1997; Woo-Sam, 1999; Nightingale et al., 2005). Cattle fed poor quality silage, which is likely to harbour *L. monocytogenes,* might shed the bacterium in faeces which could result in BTM contamination with *L. monocytogenes*. Other studies, however, failed to find an association between poor quality silage and the risk of listeriosis in farm animals (Ueno et al., 1996; Wiedmann et al., 1997; Yoshida et al., 1998). Although an assessment of feed quality was not part of our investigation, none of the feedstuffs included in this study were found to be associated with BTM contamination with *L. monocytogenes*.

Bringing cattle into the operation, which is an indicator of biosecurity, was found to increase the risk of BTM contamination with *L. monocytogenes* in the univariate analysis. Purchasing cattle from other herds as replacements or for herd expansion increases the chances of importing diseases including listeriosis. The link between this variable and the presence of *L. monocytogenes* in BTM might be indirect, acting as an indicator of more relaxed herd biosecurity and hygiene protocols.

The absence in the final model of variables such as 'use of automatic take-offs', 'cattle brought into the operation' and 'outside storage of manure – not in dry lot or pen', which were statistically significant in the univariate analysis at the 5% significance level, does not mean that they are not associated with the outcome. The construction of a multivariable model shows the effect of each variable when others are simultaneously included in the model and this process may lead to the results that differ from those obtained in the univariate analysis. Although no specific management practices were identified as risk fac-

tors for the presence of *L. monocytogenes* in BTM in the multivariable model, our results suggest that special attention should be given to larger dairies (>500 milking cows) and to those dairies located in the southeast due to their increased risk for BTM contamination with *L. monocytogenes*. It is possible that the risk factors for BTM contamination with *L monocytogenes* change by a geographical region depending on complex interactions between management practices prevalent in the area and environmental characteristics. Under this hypothesis, the prevention of BTM contamination with the pathogen should be tailored to address regional husbandry practices, herd characteristics and environmental conditions of each geographical area or country.

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References

Farber, J. M., and P. I. Peterkin, 1991: *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev.* 55, 476–511.

Hassan, L., H. O. Mohammed, P. L. McDonough, and P. L. Gonzalez, 2000: A cross-sectional study on the prevalence of *Listeria monocytogenes* and *Salmonella* in New York dairy herds. *J. Dairy Sci.* 83, 2441–2447.

Hassan, L., H. O. Mohammed, and P. L. McDonough, 2001: Farm-management and milking practices associated with the presence of *Listeria monocytogenes* in New York state dairy herds. *Prev. Vet. Med.* 51, 63–73.

Hassan, L., C. L. Guard, and H. O. Mohammed, 2002: Feeding practices associated with the presence of *Listeria monocytogenes*: a case-control study in New York state dairies. *Dairy Food Environ. Sanitation* 22, 326–332.

Husu, J. R., 1990: Epidemiological studies on the occurrence of *Listeria monocytogenes* in the feces of dairy cattle. *J. Vet. Med. B* 37, 276–282.

Husu, J. R., J. T. Seppanen, S. K. Sivela, and A. L. Rauramaa, 1990: Contamination of raw milk by *Listeria monocytogenes* on dairy farms. *J. Vet. Med. B* 37, 268–275.

Jayarao, B. M., and D. R. Henning, 2001: Prevalence of foodborne pathogens in bulk tank milk. *J. Dairy Sci.* 84, 2157–2162.

Liewen, M. B., and M. W. Plautz, 1988: Occurrence of *Listeria monocytogenes* in raw milk in Nebraska. *J. Food Prot.* 51, 840–841.

Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, M. L. Yonekura, P. Hayes, and R. Weaver, 1988: Epidemic listeriosis associated with Mexican-style cheese. *N. Engl. J. Med.* 19, 823–828.

Lovett, J., D. W. Frances, and J. M. Hunt, 1987: *Listeria monocytogenes* in raw milk: detection, incidence and pathogenesis. *J. Food Prot.* 50, 188–192.

Lunden, J., R. Tolvanen, and H. Korkeala, 2004: Human listeriosis outbreaks linked to dairy products in Europe. *J. Dairy Sci.* 87, E6–E12.

Nightingale, K. K., E. D. Fortes, A. J. Ho, Y. H. Schukken, Y. T. Grohn, and M. Wiedmann, 2005: Evaluation of farm management practices as risk factors for clinical listeriosis and fecal shedding of *Listeria monocytogenes* in ruminants. *J. Am. Vet. Med. Assoc.* 27, 1808–1814.

Sanaa, M., B. Poutrel, J. L. Menard, and F. Serieys, 1993: Risk factors associated with contamination of raw milk by *Listeria monocytogenes* in dairy farms. *J. Dairy Sci.* 76, 2891–2898.

Ueno, H., K. Yolota, T. Arai, Y. Muramatsu, H. Taniyama, T. Iida, and C. Morita, 1996: The prevalence of *Listeria monocytogenes* in the environment of dairy farms. *Microbiol. Immunol.* 40, 121–124.

USDA, 2002–2003: Dairy 2002, Part III: Reference of Dairy Cattle Health and Management Practices in the United States, 2002. USDA: APHIS: VS, CEAH, National Animal Health Monitoring Systems, Fort Collins, CO. Available at: http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/dairy/dairy02/Dairy02Pt3.pdf, (accessed 8 October 2007).

Van Kessel, J. S., J. S. Karns, L. Gorski, B. J. McCluskey, and M. L. Perdue, 2004: Prevalence of salmonellae, *Listeria monocytogenes*, and fecal coliforms in bulk tank milk on US dairies. *J. Dairy Sci.* 87, 2822–2830.

Vilar, M. J., E. Yus, M. L. Sanjuán, F. J. Diéguez, and J. L. Rodríguez-Otero, 2007: Prevalence of and risk factors for Listeria species on dairy farms. *J. Dairy Sci.* 90, 5083–5088.

Wiedmann, M., T. Arvik, J. L. Bruce, J. Neubauer, F. del Piero, M. C. Smith, J. Hurley, H. O. Mohammed, and C. A. Batt, 1997: Investigation of a listeriosis epizootic in sheep in New York state. *Am. J. Vet. Res.* 58, 733–737.

Woo-Sam, N. H., 1999: Listeriosis in a Holstein cow. *Can. Vet. J.* 40, 506–508.

Yoshida, T., Y. Kato, M. Sato, and K. Hirai, 1998: Sources and routes of contamination of raw milk with *Listeria monocytogenes* and its control. *J. Vet. Med. Sci.* 60, 1165–1168.

